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Influenza Vaccine Response in Adults Exposed to Perfluorooctanoate and Perfluorooctanesulfonate

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Abstract

Supported by several epidemiological studies and a large number of animal studies, certain polyfluorinated alkyl acids are believed to be immunotoxic, affecting particularly humoral immunity. Our aim was to investigate the relationship between the antibody response following vaccination with an inactivated trivalent influenza vaccine and circulating levels of Perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS). The study population consisted of 411 adults living in the mid-Ohio region of Ohio and West Virginia where public drinking water had been inadvertently contaminated with PFOA. They participated in a larger cross-sectional study in 2005/2006 and were followed up in 2010, by which time serum levels of PFOA had been substantially reduced but were still well above those found in the general population. Hemagglutination inhibition tests were conducted on serum samples collected preinfluenza vaccination and 21 ± 3 days postvaccination in 2010. Serum samples were also analyzed for PFOA and PFOS concentrations (median: 31.5 and 9.2 ng/ml, respectively). Questionnaires were conducted regarding the occurrence and frequency of recent (during the last 12 months) respiratory infections. Our findings indicated that elevated PFOA serum concentrations are associated with reduced antibody titer rise, particularly to A/H3N2 influenza virus, and an increased risk of not attaining the antibody threshold considered to offer long-term protection. Although the direct relationship between weakened antibody response and clinical risk of influenza is not clear, we did not find evidence for an association between self-reported colds or influenza and PFOA levels nor between PFOS serum concentrations and any of the endpoints examined.

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Supplementary Data: Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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Keywords

immunotoxicology; influenza vaccine; polyfluorinated alkyl acids; Perfluorooctanoate; perfluorooctanesulfonate

Polyfluorinated alkyl acids (PFAAs) are organofluorine compounds used in various manufacturing processes that are readily found in the environment. Perfluorooctanoate (PFOA or C8) and perfluorooctanesulfonate (PFOS) are two of the most common PFAAs. They have long half-lives, initially reported to be 3.5 years (Olsen *et al.*, 2007), and their persistence in the blood of animals and humans has been linked to a number of adverse health outcomes (Steenland *et al.*, 2010). There is evidence of widespread exposure to PFOA and PFOS in the general population although levels vary between countries and over time (Calafat *et al.*, 2007; Fromme *et al.*, 2007; Kannan *et al.*, 2004; Kato *et al.*, 2011b; Olsen *et al.*, 2012). In 2007–2008, the mean serum concentrations in the general population of the United States were 13.2 ng/ml (PFOS) and 4.1 ng/ml (PFOA) (Kato *et al.*, 2011b). Although mean serum concentrations of PFOA and PFOS have decreased in recent years in developed countries (Glynn *et al.*, 2012; Kato *et al.*, 2011b; Schroter-Kermani *et al.*, 2012), likely due to decreased production and use of PFOS and PFOA, the stability of the compounds and recent increases in serum PFOA concentrations observed in Korea and PFAAs in China (Harada *et al.*, 2010; Jin *et al.*, 2007), along with their potential human health effects, are of concern.

The Dupont Company in West Virginia had used PFOA and related substances in manufacturing processes since 1951. PFOA contamination of water supplies downwind and downstream of the Dupont plant, located near the Ohio River, has been observed since the 1980s. Although production has been drastically reduced and manufacturing processes have been changed, investigations of possible health outcomes related to this contamination continue. In 2001, a class action lawsuit was filed by residents from affected communities alleging health effects from the contaminated water supplies. As part of a class action settlement, the C8 Health Project was established to investigate possible health effects in exposed populations, and follow-up studies in a subgroup of this population were conducted in 2009–2010.

Animal and human studies have suggested PFAAs are immunotoxic, affecting particularly humoral immunity. For example, in laboratory rodent studies, exposure to either PFOS or PFOA, even at blood concentrations similar to those detected in highly exposed humans, suppressed antibody responses (DeWitt *et al*., 2008, 2012; Fair *et al.*, 2011; Yang *et al.*, 2002). Using human leukocytes, *in vitro* exposure to PFOA inhibited interleukin (IL)-4 and IL-10, cytokines which help regulate immune responses (Corsini *et al.*, 2011; Fletcher *et al.*, 2011). Grandjean *et al.* (2012) found that increased PFOA or PFOS serum concentrations were associated with reduced antibody responses to childhood diphtheria and tetanus vaccines. Similarly, a small Norwegian study recently reported a negative association between maternal PFOA and PFOS serum concentrations and rubella vaccine response, as well as an increase in certain infections in children (Granum *et al.*, 2013).

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One of the most commonly used vaccines in the developed world is the seasonal influenza vaccine. Influenza is a viral illness with the potential for serious morbidity and mortality, particularly in children and the elderly, resulting in more than 30 000 fatalities per year in the United States alone (Blanciforti, 2006). The CDC currently recommends universal influenza vaccination for all individuals older than 6 months who do not have specific contraindications (eg, severe egg allergy, history of Guillain-Barré) (NCIRD, 2011). In the United Kingdom, vaccination is recommended for those in specific high-risk groups (Department of Health, 2011). Factors associated with a reduced antibody response to influenza vaccination include older age (Goodwin *et al.*, 2006; Goronzy *et al.*, 2001; Murasko *et al.*, 2002; Weksler and Szabo, 2000; Wick and Grubeck-Loebenstein, 1997), smoking status (Cruijf, 1999), use of immunosuppressive therapies, and chronic inflammatory illnesses (Admon *et al.*, 1997; Blumberg *et al.*, 1998; Cullen *et al.*, 2012; Dengler *et al.*, 1998; Holvast *et al.*, 2009; Robertson *et al.*, 2000; Salles *et al.*, 2010; Tiu *et al.*, 2011; Vilchez *et al.*, 2002). Chronic stress has also been found to be negatively associated with vaccine antibody titers in both young and old (Pedersen *et al.*, 2009; Vedhara *et al.*, 1999).

In contrast to childhood vaccines, for which pre-existing immunity is typically low, prevaccination antibody levels to influenza can be highly variable in adults due to previous infections or vaccinations with cross-reacting epitopes. For this reason, measurement of both pre- and postvaccination antibody levels is important to establish an individual's response to a vaccine. This study offered a unique opportunity to assess the immune response to influenza vaccine in a group of residents who lived in the 6 contaminated water districts surrounding the Dupont chemical plant.

Materials and Methods

Study Design

This study was conducted in 2010/2011 as part of the C8 Science Panel Studies and was approved by the appropriate Institutional Review Board (IRB) and comply with all relevant national, state, and local regulations. The C8 Health Project initially collected data on 69 030 eligible subjects between August 2005 and August 2006. Eligible participants were those who had consumed water for at least 1 year between 1950 and December 3, 2004, while living, working, or going to school in one of the following 6 water districts: Little Hocking Water Association of Ohio; City of Belpre, Ohio; Tupper Plains–Chester District of Ohio; Village of Pomeroy, Ohio; Lubeck Public Service District of West Virginia; Mason County Public Service District of West Virginia; or private water sources within aforementioned districts and areas of documented PFOA contamination. Details of the study enrollment process, including consenting procedures, have been described elsewhere (Frisbee *et al.*, 2009). In 2010, 755 participants were recalled and invited to participate in a second interview and provide a second blood sample for PFAA analysis (Fitz-Simon *et al.*, 2013). This interview included questions regarding the occurrence and frequency of a number of recent (during the last 12 months) common infections, including coughs, colds, flu, and other upper respiratory infections. Frequency information was only gathered for cold episodes but not influenza specifically. A subset of these participants who had not yet

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received the annual flu vaccine were offered the vaccine and asked to consent to pre- and postvaccination blood sampling to determine virus-specific antibody titers.

The study was conducted with every participant receiving an influenza vaccine and short health questionnaire. The prevaccination serum sample was collected at the time of vaccination, and the postvaccination sample was collected 21 ± 3 days later (FDA, 2007). Subjects (411) were healthy adults aged over 18 years who did not have a history of influenza vaccination in the past 3 months or a relatively high risk of influenza complications (cancer, recent history of influenza, and vaccine allergy).

Vaccine

A single lot of FLUVIRIN (Novartis, Cambridge, Massachusetts), an inactivated trivalent vaccine containing influenza serotypes A/H3N2 and A/H1N1 (swine flu) and influenza B, was used in the study.

Hemagglutination Inhibition Assays

Sera were tested for influenza-specific antibody by a hemagglutination inhibition (HI) assay using turkey red blood cells (tRBCs) for A/H3N2 and A/H1N1 and influenza B. Nonspecific HI activity was removed by treating the serum samples with receptor destroying enzyme (RDE, ATCC) overnight at 4°C. The RDE-treated sera were tested for residual HI activity by adding 50 µl of the treated serum to 50 µl of the 0.5% tRBCs. This mixture was incubated for 30–60 min and then read to determine whether HI activity was present. If HI activity was present, the nonspecific agglutination inhibitors were removed by adsorption of the serum with packed tRBCs for 1 h at 4°C. Patient serum was prepared by using a 2-fold dilution curve starting at 1:10 and ending at 1:10 240. The serum dilution series (25 µl/dilution) was mixed with 25 µl of optimized dilutions of the 3 influenza antigens in a V-bottom 96-well plate and incubated for 45–60 min at 25°C. Fifty microliters of standardized 0.5% tRBCs were then added to the appropriate wells and incubated at room temperature for 30–60 min to allow the RBCs to settle to the bottom of the well. Positive (known titer of influenza-specific antibodies) and negative (no influenza-specific antibodies) control sera were run in parallel to the test subject sera. Quality control plates were prepared to confirm the optimized antigen. If there were no hemagglutinins present in the sample, as in the control wells that did not contain antigen, the tRBC pellet would stream across the bottom of the well forming a distinct “teardrop” shape. The plates were read when the “teardrop” in the control wells (no antigen) extended from the middle of the well to the outer edge of the well. If influenza-specific antibody was present in the serum sample, the influenza antigen would be bound by the antibody before it could interact with the tRBCs to cause agglutination. The dilution of serum at which a complete “teardrop” formed was designated at the influenza-specific antibody titer for the antigen being tested.

PFOA and PFOS Concentrations

Laboratory measurement of PFOA and PFOS used online solid phase extraction coupled with reversed-phase high-performance liquid chromatography separation and detection by isotope-dilution tandem mass spectrometry (Kato *et al.*, 2011a). The detection limits were 0.5 ng/ml (PFOA) and 0.2 ng/ml (PFOS). At the laboratory, interday precision, calculated as

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the relative standard deviation of 60 repeated measurements in a 6-month period, was below 8% (PFOA: 7.6% [3.1 ng/ml] and 5.8% [11.7 ng/ml]; PFOS: 7.3% [8.2 ng/ml] and 7.6% [27.5 ng/ml]). Intraday precision, calculated as the relative standard deviation of 5 repeated measurements within 1 day, ranged from approximately 2% to 6% (PFOA: 2.8% [3.1 ng/ml] and 1.7% [11.7 ng/ml]; PFOS: 5.8% [8.2 ng/ml] and 4.9% [27.5 ng/ml]).

Analysis

Vaccine response measures—The geometric mean antibody titer (GMT) rises for different participant characteristics are described. Statistical significance between participant groups for GMTs was determined by examining the bounds of the 2-sided 95% confidence intervals to determine whether they overlapped. To explore the associations between antibody titers and PFOA and PFOS levels, several measures of vaccine response were used. Firstly, the antibody titer rise following vaccination was calculated by subtracting the prevaccination titer from the postvaccination titer. Secondly, the ratio of pre- and postvaccination titers was examined by dividing the postvaccine titer by the prevaccine titer. The antibody titer rise and ratio for antibodies specific for each strain showed skewed distributions and were $\log_{10}$ transformed for the analysis.

Additional analyses used dichotomous surrogate outcomes to measure vaccine response. The associations between PFOA/PFOS and seroconversion, defined as a 4-fold or greater increase in antibody titer following vaccination, and seroprotection, defined as a postvaccination HI titer > 1:40 (irrespective of prevaccination levels) were examined. The seroprotection threshold is usually considered indicative of protection from a clinical testing perspective (Beyer *et al.*, 2004).

Exposure measures—Concentrations of PFOA and PFOS showed skewed distribution and were $\log_{10}$ transformed. PFOA and PFOS were examined as linear variables in both the transformed and untransformed form and grouped as categorical exposure groups of quartiles of PFOA and PFOS levels.

Regression analysis—The associations between the antibody titer rise or ratio and untransformed, $\log_{10}$ transformed and concentration quartiles of PFOA and PFOS were examined using linear regression. Given the significant skew in the PFOA measurements, only $\log_{10}$-transformed and quartile concentrations of PFOA and PFOS are presented in the main text. Initially, each association was examined considering only PFOA or PFOS as an exposure (unadjusted results for linear models only are shown). Age and gender were then included as obligatory covariates. The effect of age on antibody titer rise and ratio was found to be nonlinear; therefore, age was fitted using a restricted cubic spline function with 7 knots in the linear regression model. The possible effect of additional *a priori* confounders including smoking status, any previous influenza vaccination (participants were excluded if this had occurred in the last 3 months), specific H1N1 vaccination in the previous year, day of serum sample collection, coexisting medical conditions and common anti-inflammatory, and pain relief medications was also considered. Potential confounders associated with the $\log_{10}$-transformed antibody titer rise or antibody titer ratio of that vaccine strain ($p < .20$) were tested in the model and retained if they remained independently associated with the

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outcome ($p < .05$). The confounders included in the final linear regression models were age (fitted as cubic spline), gender, mobility (as measured by the number of addresses since 1970 or birth), and a history of previous influenza vaccination.

Multivariable logistic regression models were used to calculate odds ratios to assess the effect of PFOA and PFOS on the likelihood of achieving seroconversion or seroprotection following vaccination. The odds ratio represents a close approximation of relative risk. *A priori* confounders associated with seroconversion or seroprotection ($p < .20$) in univariate analysis were included in a multivariate regression model and retained if they remained independently associated with the outcome ($p < .05$). In the logistic regression model, age was modeled as a categorical variable in 10-year age bands. Only adjusted models are presented in the text.

Multivariable logistic regression models were also used to calculate odds ratios to assess the effect of serum PFAA levels on the likelihood of influenza and colds in the past year. Because the symptoms of influenza are not readily distinguished from other respiratory infections (Call *et al.*, 2005), both self-reported colds and influenza were considered in this analysis. Ordered multi-variable logistic regression was used to test whether there was a risk between increasing frequency of cold and influenza episodes during the year and increased PFAA levels. Models for the occurrence of adult influenza were first fitted adjusted for age and sex only. The possible effect of other *a priori* con-founders including smoking status, alcohol intake, body mass index, diagnosis of diabetes, and educational level was also considered but was not found to significantly affect the final model. All statistical analysis was conducted using Stata 12.1 (StataCorp, Texas).

Results

Baseline

A total of 411 adults had a prevaccination antibody titer taken and received the influenza vaccination as part of the study (Table 1). The population median $\log_{10}$ serum PFOA concentration was 1.50 (IQR: 1.14, 1.95) and PFOS, 0.96 (IQR: 0.76, 1.16), reflecting a geometric mean concentration of 33.74 ng/ml (95% confidence interval [CI]: 29.78, 38.22) and 8.32 ng/ml (95% CI: 7.65, 9.05), respectively. There was a modest trend of higher PFOA and PFOS concentrations among older participants (Table 1). Postvaccination serology was available for 403 (98.1%) participants on whom most analysis is based. Participants had higher prevaccination antibody GMTs for A/H3N2 than for A/H1N1 and B (20.69 [95% CI: 17.87, 23.95], 16.11 [95% CI: 13.90, 18.68], and 8.72 [95% CI: 8.02, 9.49]), respectively (not shown). Prevaccination GMTs were higher among those who reported previous influenza vaccination from last year than those who did not (10.17, 19.81, and 31.42 compared with 6.04, 9.83, and 7.60 for B, A/H1N1, and A/H3N2 antigens, respectively), and there was a tendency toward greater pre-existing immunity (prevaccination titers) among those who reported greater residential mobility (a higher number of previous addresses) (Table 1). Following vaccination, there was strong evidence of a larger response to A/H1N1 (overall GMT rise 343.08 [95% CI: 296.07, 397.56]) compared with A/H3N2 (151.49 [95% CI: 126.19, 181.86]) or B (44.21 [95% CI: 38.58, 50.66]). Furthermore, the antibody response to A/H1N1 was strongly associated with age,

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being higher among younger participants (Supplementary Table S1). For Flu B, a higher antibody response was seen among men than women (54.47 [95% CI: 44.92, 66.05] and 35.32 [95% CI: 29.26, 42.64], respectively), and for both A/H1N1 and B, there was strong evidence that those who reported previous influenza vaccination had lower antibody responses than those who did not (34.91 and 296.68 compared with 74.41 and 467.28 for B and H1N1, respectively) (Supplementary Table S1).

Details of prevaccination antibody GMTs by medical comorbidity and medication groups can be found in Supplementary Table S2. Participants with a history of asthma had a higher prevaccination GMT for H1N1 than those without, reflecting the higher rate of previous A/H1N1 vaccination reported among those with asthma (65% vs 35.2%, $p = .03$ [not shown on table]). Similarly, those with diabetes reported both higher rates of previous influenza vaccination and had a higher prevaccination GMT for A/H3N2 than those without diabetes (41.38 [95% CI: 25.37, 67.48] vs 19.16 [95% CI: 16.45, 22.30]).

Vaccine Response

GMT rise—Vaccine response as measured by the GMT rise against each of the 3 vaccine components is shown in Table 2 by quartile of PFAA. Individuals in the fourth quartile of PFOA exposure had a lower GMT rise in Flu B; however, this trend was less evident for A/H1N1 and PFOA. For other A/H3N2 and PFOA and for any type with PFOS, there was no evident pattern with rise in antibody titers.

Log_{10}-transformed antibody titer rise and ratio—There was a negative association between log_{10}-transformed A/H3N2 antibody titer ratio and PFOA when log_{10}-transformed PFOA was fitted as a continuous variable in the regression model (adjusted coefficient −0.12 [95% CI: −0.25, 0.02, $p = .09$]) (Table 3). By PFOA quartile, the A/H3N2 antibody titer ratio showed a largest reduction in response for the highest quartile of PFOA concentrations (adjusted coefficient −0.22 [95% CI: −0.43, −0.01]) (Table 3).

For titer rise, there was no overall trend with exposure, but the second and third quartiles of PFOA concentration had lower rises in log_{10}-transformed A/H3N2 antibody titers than those in the first quartile (adjusted coefficients −0.28 [95% CI: −0.51, −0.06] for second quartile and −0.37 [95% CI: −0.60, 0.13], respectively) (Table 3).

Although unadjusted results suggested a negative association between log_{10}-transformed A/H1N1 antibody titer rise with quartiles of PFOA concentrations, both PFOA blood concentrations and A/H1N1 antibody response are related to age, and this association did not persist in the adjusted model (Table 3). The effects on A/H3N2 antibody titers were not confounded by age. There was no suggestion from the linear regression analysis of an association between influenza B antibody titer rise or ratio and PFOA or PFOS concentrations (Table 3).

Seroconversion/seroprotection—Seroconversion for influenza vaccines was defined as having a 4-fold increase in antibody titer. Seroprotection was defined as an HI antibody titer ≥ 1:40 postvaccination independent of the prevaccine HI titer. Both are used as evidence of efficacy to help support the licensing of seasonal inactivated influenza vaccines

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(FDA, 2007). The proportions of participants who seroconverted following vaccination and associations with PFAA are shown in Table 4. Overall, a higher proportion of participants seroconverted to H1N1 than H3N2 and B (84.12% [95% CI: 80.54, 87.70] compared with 64.76% [60.08, 69.45] and 62.03% [57.28, 66.79] for H3N2 and B antigens, respectively). As expected, previous influenza vaccination was associated with a decreased likelihood of seroconverting to all 3 components of the vaccine (ORs 0.25, 0.13, and 0.31 for B, A/H1N1, and A/H3N2, respectively; $p < .01$ for all) (Supplementary Table S4). Increased age was also associated with decreased odds of seroconverting to influenza B ($p = .04$) and a higher proportion of men seroconverted to B than women (68.3% vs 55.6) (Supplementary Table S4). There was weak evidence of odds of seroconversion to A/H1N1 increasing with PFOA concentration with the odds ratio rising to 2.23 (0.90–5.53) in the fourth quartile, but all confidence intervals were wide, and we observed no other associations of note between PFOA or PFOS and likelihood of seroconverting to the vaccine components.

The proportions of participants who were seroprotected (HI titer ≥ 40) at their postvaccination blood test were 95.5% for A/H1N1, 83.9% for A/H3N2, and 66% for B. (Table 5). Unlike B and A/H3N2, the response to A/H1N1 was not strongly associated with whether the participant had received a previous influenza vaccination (Supplementary Table S5). Participants who reported previous influenza vaccination had a decreased likelihood of seroprotection for B (odds ratio [OR]: 0.50 [95% CI: 0.29, 0.84], $p = .01$), but an increased likelihood of seroprotection to A/H3N2 (OR: 2.09 [95% CI: 1.18, 3.69], $p = .01$) (Supplementary Table S5). Increasing age was also strongly associated with decreased seroprotection for B and H1N1 (Supplementary Table S5).

After adjustment for age, gender, and past vaccination, there was some evidence of seroprotection for decreased likelihood of seroprotection from A/H3N2 in relation to PFOA (by PFOA quartile the odds ratios were 0.34 [0.14, 0.83], $p = .02$, 0.28 [0.11, 0.70], $p = .01$, and 0.39 [0.15, 0.99], $p = .05$, respectively). Conversely, there was a suggestion of an increasing trend of seroprotection for A/H1N1, with an overall OR of 2.34 (0.91–6.07) per log unit of PFOA. No other strong associations between seroprotection and PFOA or PFOS levels were found.

Self-reported cold and influenza episodes—Data were available on the frequency of respiratory infections in the population of the entire follow-up study ($n = 755$), which included those who participated in the vaccination study. One hundred and sixty-three (21.6%) adult participants reported an episode of influenza in the 12 months preceding the questionnaire, whereas 538 (71.3%) reported a cold in the same time period (Tables 6 and 7). Age was a major confounder with younger participants who more likely to report colds and flu and more likely to have had lower PFAA levels (Table 1). Adjusted models assessed either colds or flu, comparing those with and without a reported infection in relation to either log PFAA as a continuous variable or quartiles of PFAA concentration. For colds, the frequency of reported episodes was also assessed. There was no evidence for any association between PFAA concentrations and either self-reported colds or flu (Tables 6 and 7).

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Discussion

This study offered a unique opportunity to help determine whether PFAA concentrations affected the humoral antibody response in humans as determined by HI titers following adult seasonal influenza vaccine. The opportunity to determine pre-and postvaccination HI titers allowed 4 measures of immune response—the rise, the ratio, seroconversion, and seroprotection—to be evaluated in relation to concurrent measured serum concentrations of PFOA and PFOS. This design is particularly valuable in the assessment of influenza vaccines, which are designed to protect against commonly circulating viruses, and in adults, for whom immune function is a product of the recent study vaccine response, prior vaccinations, and pre-existing viral exposures. This design allowed assessment of the association of circulating levels of PFAA and the humoral immune response following immunization to 3 influenza subtypes relative to previous influenza-specific antibody levels and also with the attainment of a relative protection antibody threshold. It is important to note that HI titers in vaccine studies serve only as a surrogate for protection from influenza illness or disease, and the absolute antibody titer that confers protection has not been defined.

Age was found to be highly correlated with both pre-existing immunity and vaccine response to some but not all of the subtypes examined. The finding of decreasing antibody response to influenza vaccination with increasing age and history of influenza vaccination in previous years is consistent with a number of studies of influenza vaccine response. Age was carefully adjusted for by using a cubic spline term for age in the adjusted linear regression models. However, the higher vaccine response to influenza B found among men has not been reported elsewhere. This gender difference in the study was isolated to this virus serotype and is not consistent with other published studies (Cook, 2008; Engler *et al.*, 2008; Klein *et al.*, 2010), suggesting that this may be a chance finding.

The most consistent finding in relation to the 2 exposures considered and the 3 flu strains was evidence of a reduced antibody response to A/H3N2 influenza vaccine by higher PFOA concentration, refected in the results for titer rise, titer ratio, and seroprotection, though not seroconversion. confidence intervals were relatively wide, especially when separated into quartile groups, but some results, most notably the reduction in seroprotection, were significantly lower in higher serum PFOA concentration quartiles. These results suggest that individuals with raised PFOA concentrations have an increased risk of not attaining the antibody threshold considered to offer long-term protection from this virus strain. There were no other consistent associations, the only other associations coming close to statistical significance being increases in immune response in for A/H1N1 in relation to PFOS (titer rise) and PFOA (seroconversion and seroprotection, top quartile only).

Previous animal and human studies have suggested PFAAs are associated with a general suppression of humoral immune response; however, we only observed strong evidence of suppression to the A/H3N2 vaccine component. The differences in the ability of the various influenza strains to show a significant effect in relationship to PFOA concentrations may be due to differences in their antigenic determinants. Those strains with more conserved antigenic determinants (epitopes) would likely have a more significant memory cell

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response. It is generally thought that memory cell responses are less sensitive to chemical immunosuppression than the primary, and thus, response to strains containing more conserved antigenic determinants than other strains might be less likely to show an effect or at least behave kinetically different in their immune response due to a more significant memory cell response. Nonetheless, the lack of consistency between the various endpoints increases the plausibility that the results are due to chance.

In addition to age, a number of medical comorbidities and medications were also considered as potential contributors to, or confounders of, vaccine response; however, the regression models failed to find any evidence of these associations, likely due in part to the low prevalence of these factors in this population. A potential limitation in the study was the reporting of previous vaccination. Analysis revealed the strong effect of previous influenza vaccination on immune response. This information was self-reported by participants using a questionnaire. Without validation, this factor has the potential for recall bias, and the variation in seasonal influenza vaccine year to year would alter the degree to which previous vaccination infers pre-existing immunity against the study vaccine. However, we consider it unlikely that any problems with recall of past vaccinations would be associated with PFAA category.

The reduced influenza vaccine response observed in this study in adults with elevated PFOA concentrations is of note given the findings reported previously in children by Grandjean *et al.* (2012) and Granum *et al.* (2013) showing similar effects. In addition to the differences in the ages of the participants, the most significant difference between our study and theirs was in the experimental design. The latter studies collected serum for analysis only prior and not following booster immunizations. Boosters for childhood vaccinations are normally conducted at least 3 years or more following the initial immunization to stimulate memory cell responses. Hence, significant decay of the primary antibody response would have occurred as peak IgG levels appear 21 days postimmunization. Swartz *et al.* (2003) in a large study monitoring vaccine titers in children following infant immunization showed that the antibody titer decays by > 90% from the primary immunization within 3 years prior to booster. Thus, the titers reported from the previous PFAA studies in children represent differences detected from residual antibody and not the peak response that we monitored. On the other hand, participants in our study had prevaccination titers for all vaccine viruses, especially A/H1N1 which would not have been expected following infant immunization. This pre-existing immunity may have limited the power of our study to capture differences between exposure groups in antibody titer rises.

Our study found no association between PFAA concentration and recent self-reported cold or influenza episodes. It is possible that the extent of suppression in the vaccine response associated with PFOA exposure is insufficient to change in an individual's risk of infections, particularly in a small population. Given the high background rate of respiratory infections, with over 70% of adults reported having experienced an episode in the preceding 12 months, there is not much scope to detect an increase in infections. Furthermore, self-report respiratory infection, particularly influenza, has poor specificity and sensitivity, particularly during times of pandemic virus circulation and subsequent heightened public awareness (Jutel *et al.*, 2011). During the 2009/2010 influenza season in the United States, the main

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circulating virus was the 2009 pandemic influenza A (H1N1), and very few other seasonal influenza viruses were detected (CDC, 2010). Therefore, if the impact of the A/H3N2 strain of influenza specifically was affected by PFOA serum concentrations, it is plausible that there was too little of this virus type circulating to detect this association in self-reported influenza cases.

In any case, our findings provide evidence that PFOA at serum concentrations between 13.7 and 90 ng/ml, about 4- to 5-fold above the current levels found in the general U.S. population (Kato *et al.*, 2011b), are associated with a reduced HI response to A/H3N2 influenza virus, a commonly occurring flu virus. We found no evidence of an association between self-reported colds or influenza and PFAA concentrations. Furthermore, we saw no evidence that PFOS serum concentrations are associated with reduced vaccine responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline Participant Characteristics, PFOA/PFOS Serum Concentrations (ng/ml), and Prevaccination GMTs (*n* = 411)

	Number (%)	Median Log_{10} PFOA Serum Concentration at Vaccination (IQR)	Median Log_{10} PFOS Serum Concentration at Vaccination (IQR)	Influenza Type B GMT (95% CI)	Influenza A H1N1 GMT (95% CI)	Influenza A H3N2 GMT (95% CI)
Gender						
Female	200 (48.7)	1.37 (1.03, 1.77)	0.84 (0.64, 1.01)	9.36 (8.28, 10.58)	16.64 (13.56, 20.43)	26.21 (21.35, 32.17)
Male	211 (51.3)	1.64 (1.28, 2.03)	1.09 (0.91, 1.23)	8.16 (7.26, 9.17)	15.63 (12.62, 19.36)	16.53 (13.46, 20.31)
Age (years)						
< 30	49 (11.9)	1.43 (1.05, 1.66)	0.93 (0.82, 1.05)	8.80 (6.79, 11.41)	18.37 (12.37, 27.28)	16.88 (10.76, 26.47)
31–40	107 (26.0)	1.23 (1.03, 1.79)	0.89 (0.64, 1.09)	9.13 (7.63, 10.93)	19.62 (14.09, 27.30)	18.87 (14.30, 24.88)
41–50	109 (26.5)	1.54 (1.15, 1.81)	0.95 (0.74, 1.14)	8.81 (7.56, 10.26)	18.18 (13.17, 25.09)	16.74 (12.57, 22.30)
51–60	107 (26.0)	1.59 (1.26, 2.05)	1.04 (0.84, 1.21)	8.50 (7.24, 9.99)	12.71 (10.01, 16.13)	25.75 (19.43, 34.13)
> 60	39 (9.5)	1.83 (1.65, 2.26)	1.09 (0.92, 1.35)	7.94 (5.87, 10.74)	10.93 (7.45, 16.03)	34.09 (20.28, 57.29)
Current smoker						
No	331 (80.5)	1.52 (1.14, 1.96)	0.96 (0.76, 1.17)	8.49 (7.73, 9.34)	15.52 (13.17, 18.30)	21.39 (18.17, 25.17)
Yes	80 (19.5)	1.45 (1.11, 1.73)	0.96 (0.76, 1.12)	9.74 (8.08, 11.75)	18.82 (13.36, 26.52)	18.03 (12.84, 25.31)
Any previous influenza vaccine (not within last 3 months)						
No	121 (29.4)	1.46 (1.15, 1.90)	1.05 (0.79, 1.22)	6.04 (5.49, 6.64)	9.83 (7.99, 12.10)	7.60 (6.51, 8.87)
Yes	290 (70.6)	1.52 (1.13, 1.98)	0.94 (0.74, 1.13)	10.17(9.12, 11.33)	19.81 (16.44, 23.87)	31.42 (26.34, 37.48)
H1N1 vaccine in last influenza season						
No	181 (62.4)	1.56 (1.16, 2.03)	0.96 (0.77, 1.14)	9.85 (8.63, 11.24)	11.61 (9.74, 13.84)	26.25 (21.03, 32.76)
Yes	108 (37.2)	1.44 (1.11, 1.84)	0.91 (0.72, 1.12)	10.59(8.76, 12.81)	47.57 (33.61, 67.32)	42.65 (31.93, 56.97)
Don't know	1 (0.3)	0.82 (0.82, 0.82)	0.04 (0.04, 0.04)	40.00 (—, —)	160.00 (—, —)	20.00 (—, —)
Mobility (number of addresses since birth or 1970 if born prior to 1970)						
1–3	56 (13.6)	1.79 (1.40, 2.11)	1.06 (0.86, 1.26)	7.62 (6.11, 9.49)	12.50 (8.51, 18.36)	21.28 (14.65, 30.91)
4–6	205 (49.9)	1.49 (1.17, 1.86)	0.94 (0.76, 1.16)	8.65 (7.73, 9.68)	15.42 (12.52, 18.98)	20.76 (16.86, 25.55)
7–9	87 (21.2)	1.43 (1.03, 1.86)	0.96 (0.79, 1.17)	8.33 (6.77, 10.24)	17.33 (12.41, 24.19)	17.47 (12.72, 23.98)
≥ 10	63 (15.3)	1.35 (1.03, 1.82)	0.94 (0.67, 1.11)	10.80 (8.58, 13.60)	21.13 (14.21, 31.43)	25.20 (16.64, 38.15)
PFOA and PFOS serum concentrations at time of vaccination						
PFOA[a]						

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	Number (%)	Median Log_{10} PFOA Serum Concentration at Vaccination (IQR)	Median Log_{10} PFOS Serum Concentration at Vaccination (IQR)	Influenza Type B GMT (95% CI)	Influenza A H1N1 GMT (95% CI)	Influenza A H3N2 GMT (95% CI)
First quartile	103 (25.1)	0.93 (0.67, 1.04)	0.75 (0.45, 0.93)	9.60 (8.04, 11.47)	18.95 (14.01, 25.64)	23.99 (18.09, 31.80)
Second quartile	103 (25.1)	1.32 (1.21, 1.42)	0.99 (0.76, 1.20)	7.95 (6.85, 9.24)	18.08 (13.27, 24.64)	17.13 (12.73, 23.05)
Third quartile	103 (25.1)	1.69 (1.60, 1.81)	1.04 (0.81, 1.20)	8.34 (7.07, 9.84)	16.57 (12.14, 22.60)	15.49 (11.98, 20.02)
Fourth quartile	102 (24.8)	2.17 (2.07, 2.34)	1.09 (0.91, 1.25)	9.09 (7.54, 10.96)	11.85 (9.10, 15.43)	28.87 (20.75, 40.15)
PFOS[b]						
First quartile	106 (25.8)	1.11 (0.75, 1.44)	0.58 (0.34, 0.67)	9.87 (8.34, 11.69)	17.21 (12.75, 23.23)	21.92 (16.17, 29.70)
Second quartile	101 (24.6)	1.57 (1.11, 2.00)	0.88 (0.83, 0.92)	8.96 (7.55, 10.64)	15.95 (11.77, 21.60)	23.26 (17.58, 30.78)
Third quartile	102 (24.8)	1.62 (1.25, 2.05)	1.06 (1.02, 1.10)	8.16 (6.86, 9.70)	17.94 (13.09, 24.59)	22.15 (16.32, 30.04)
Fourth quartile	102 (24.8)	1.72 (1.43, 2.12)	1.27 (1.21, 1.38)	7.99 (6.76, 9.45)	13.67 (10.39, 17.99)	16.20 (12.13, 21.65)

Note. Range of PFAA concentrations within quartiles:

[a] PFOA: First quartile: 0.25–13.7 ng/ml; second quartile: 13.8–31.5 ng/ml; third quartile: 31.6–90 ng/ml; fourth quartile: 90.4–2140 ng/ml.

[b] PFOS: First quartile: 0.1–5.8 ng/ml; second quartile: 5.9–9.2 ng/ml; third quartile: 9.3–14.5 ng/ml; fourth quartile: 14.7–42.3 ng/ml.

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Table 2

GMT Rise Following Vaccination (95% CI) by Quartile of PFOA/PFOS Serum Concentration (*n* = 403)

	Influenza Type B	Influenza Type A H1N1	Influenza A H3N2
	GMT (95% CI)	**GMT (95% CI)**	**GMT (95% CI)**
PFOA and PFOS serum concentrations at time of vaccination			
PFOA[a]			
First quartile	49.46 (38.14, 64.12)	476.23 (360.77, 628.65)	228.86 (161.53, 324.27)
Second quartile	46.00 (35.28, 59.97)	352.22 (255.33, 485.88)	125.36 (86.01, 182.73)
Third quartile	43.55 (33.08, 57.33)	306.32 (232.58, 403.44)	104.13 (72.47, 149.64)
Fourth quartile	**20.90 (16.58, 28.24)**	274.79 (202.85, 372.23)	183.73 (127.28, 265.23)
PFOS[b]			
First quartile	42.25 (33.41, 53.42)	342.31 (256.04, 457.65)	137.69 (98.70, 192.09)
Second quartile	41.48 (30.73, 55.99)	280.44 (197.64, 397.92)	147.27 (99.27, 218.47)
Third quartile	41.13 (31.65, 53.44)	417.74 (318.99, 547.07)	211.02 (141.24, 315.27)
Fourth quartile	52.83 (38.92, 71.71)	341.82 (258.03, 452.81)	126.69 (88.85, 180.65)

Note. Bold values represent significant associations. Range of PFAA concentrations within quartiles:

[a]PFOA: First quartile: 0.25–13.7 ng/ml; second quartile: 13.8–31.5 ng/ml; third quartile: 31.6–90 ng/ml; fourth quartile: 90.4–2140 ng/ml.

[b]PFOS: First quartile: 0.1–5.8 ng/ml; second quartile: 5.9–9.2 ng/ml; third quartile: 9.3–14.5 ng/ml; fourth quartile: 14.7–42.3 ng/ml.

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Table 3

Linear regression Coefficients of Log_{10}-Transformed influenza Antibody Titer rise and Log_{10}-Transformed influenza Antibody Titer ratio With unit increase in Log_{10} Transformed and Quartiles of PFOA and PFOS Serum Concentration (n = 403)

	Log PFOA (ng/ml)			Log PFOS (ng/ml)		
	Regression Coefficient	**95% CI**	***p* Value**	**Regression Coefficient**	**95% CI**	***p* Value**
a. Log_{10}-transformed PFOA/PFOS (as continuous variable)						
Influenza type B						
Log_{10}-transformed antibody titer rise (n = 359)[a]						
Unadjusted	−.05	(−0.16, 0.05)	.33	.09	(−0.07, 0.24)	.29
Adjusted[b]	−.02	(−0.13, 0.09)	.73	.05	(−0.11, 0.21)	.56
Log_{10}-transformed antibody titer ratio (postvaccine: prevaccine)						
Unadjusted	−.05	(−0.15, 0.05)	.30	.10	(−0.04, 0.25)	.16
Adjusted[b]	−.02	(−0.11, 0.08)	.73	.05	(−0.09, 0.18)	.52
Influenza A/H1N1						
Log_{10}-transformed antibody titer rise (n = 322)[a]						
Unadjusted	−.11	(−0.22, 0.01)	.07	.09	(−0.08, 0.25)	.32
Adjusted[b]	−.03	(−0.14, 0.09)	.63	.15	(−0.02, 0.32)	.08
Log_{10}-transformed antibody titer ratio (postvaccine: prevaccine)						
Unadjusted	.02	(−0.12, 0.15)	.79	.12	(−0.07, 0.32)	.22
Adjusted[b]	.07	(−0.06, 0.21)	.30	.10	(−0.11, 0.30)	.36
Influenza A/H3N2						
Log_{10}-transformed antibody titer rise(n = 372)[a]						
Unadjusted	−.02	(−0.16, 0.13)	.81	.08	(−0.13, 0.28)	.47
Adjusted[b]	−.01	(−0.17, 0.14)	.86	.09	(−0.13, 0.32)	.42
Log_{10}-transformed antibody titer ratio (postvaccine: prevaccine)						
Unadjusted	−.15	(−0.28, −0.02)	.03	.04	(−0.15, 0.24)	.68
Adjusted[b]	−.12	(−0.25, 0.02)	.09	−.005	(−0.20, 0.19)	.96

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	Log PFOA (ng/ml)			Log PFOS (ng/ml)		
	Regression Coefficient	**95% CI**	***p* Value**	**Regression Coefficient**	**95% CI**	***p* Value**
	PFOA Quartiles (ng/ml)[c]			PFOS Quartiles (ng/ml)[d]		
	Regression Coefficient	95% CI	*p* Value	Regression Coefficient	95% CI	*p* Value
b. Quartiles of PFOA/PFOS concentration						
Influenza type B						
Log_{10}-transformed antibody titer rise (n = 359)[e]						
Adjusted[b]						
First quartile	Referent			Referent		
Second quartile	−.03	(−0.19, 0.13)	.69	.02	(−0.13, 0.18)	.76
Third quartile	−.02	(−0.19, 0.15)	.82	−.03	(−0.19, 0.14)	.73
Fourth quartile	−.07	(−0.24, 0.10)	.42	.04	(−0.14, 0.21)	.68
Log_{10}-transformed antibody titer ratio						
Adjusted[b]						
First quartile	Referent			Referent		
Second quartile	.05	(−0.09, 0.19)	.53	.004	(−0.14, 0.14)	.96
Third quartile	.07	(−0.07, 0.22)	.32	−.02	(−0.16, 0.12)	.78
Fourth quartile	−.03	(−0.17, 0.12)	.71	.03	(−0.12, 0.18)	.71
Influenza A/H1N1						
Log_{10}-transformed antibody titer rise (n = 322)[e]						
Adjusted[b]						
First quartile	Referent			Referent		
Second quartile	−.09	(−0.27, 0.08)	.31	−.04	(−0.21, 0.14)	.68
Third quartile	−.10	(−0.28, 0.09)	.30	.13	(−0.04, 0.31)	.14
Fourth quartile	−.12	(−0.30, 0.06)	.19	.10	(−0.09, 0.29)	.30
Log_{10}-transformed antibody titer ratio						
Adjusted[b]						
First quartile	Referent			Referent		
Second quartile	−.08	(−0.29, 0.12)	.43	−.07	(−0.28, 0.13)	.47

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	Log PFOA (ng/ml)			Log PFOS (ng/ml)		
	Regression Coefficient	**95% CI**	***p* Value**	**Regression Coefficient**	**95% CI**	***p* Value**
Third quartile	−.04	(−0.25, 0.18)	.72	.03	(−0.18, 0.24)	.78
Fourth quartile	.07	(−0.14, 0.29)	.51	.03	(−0.19, 0.26)	.77
Influenza A/H3N2						
Log_{10}-transformed antibody titer rise (n = 372)[e]						
Adjusted[b]						
First quartile	Referent			Referent		
Second quartile	−.28	(−0.51, -0.06)	.02	.03	(−0.19, 0.26)	.78
Third quartile	−.37	(−0.60, −0.13)	.002	.18	(−0.06, 0.41)	.14
Fourth quartile	−.12	(−0.36, 0.13)	.35	−.04	(−0.28, 0.21)	.77
Log_{10}-transformed antibody titer ratio						
Adjusted[b]						
First quartile	Referent			Referent		
Second quartile	−.10	(−0.30, 0.10)	.31	−.06	(−0.26, 0.14)	.56
Third quartile	−.07	(−0.28, 0.14)	.49	.02	(−0.18, 0.23)	.84
Fourth quartile	−.22	(−0.43, -0.01)	.04	−.03	(−0.24, 0.19)	.82

[a] *Note*. Log_{10}-transformed antibody titer rise missing for participants with untransformed antibody titer rise of 0.

[b] Adjusted for age (cubic spline), gender, mobility, and history of previous influenza vaccination.

Range of PFAA concentrations within quartiles:

[c] PFOA: First quartile: 0.25–13.7 ng/ml; second quartile: 13.8–31.5 ng/ml; third quartile: 31.6–90 ng/ml; fourth quartile: 90.4–2140 ng/ml.

[d] PFOS: First quartile: 0.1–5.8 ng/ml; second quartile: 5.9–9.2 ng/ml; third quartile: 9.3–14.5 ng/ml; fourth quartile: 14.7–42.3 ng/ml.

[e] Log_{10}-transformed antibody titer rise missing for participants with untransformed antibody titer rise of 0.

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Table 4

Logistic Regression OR (95% CI) of Seroconversion (4-Fold increase in Antibody Titer Following Vaccination) for Log_{10} Transformed and Quartiles of PFOA and PFOS Concentration (ng/ml) (*n* = 403)

	Number Who Seroconvert Following Vaccination (Row%)	Adjusted OR (95% CI)[a]	*p* Value	*p* Value for Heterogeneity Across Quartiles	*p* Value for Trend Over Quartiles
Influenza type B—odds ratio for seroconversion					
Log_{10} transformed (as continuous variable, odds ratio per unit rise in PFOC measure)					
Log_{10} PFOA	250/403 (62.0%)	0.80 (0.53, 1.21)	.30		
Log_{10} PFOS	250/403 (62.0%)	1.17 (0.63, 2.17)	.63		
Quartiles (as categorical variable)					
PFOA[a]					
First quartile	61/100 (61.0%)	1		.09	.28
Second quartile	71/102 (69.6%)	1.43 (0.76, 2.70)	.26		
Third quartile	66/100 (66.0%)	1.39 (0.73, 2.66)	.32		
Fourth quartile	52/101 (51.5%)	0.71 (0.38, 1.36)	.30		
PFOS[b]					
First quartile	67/104 (64.4%)	1		.76	.75
Second quartile	54/97 (55.7%)	0.72 (0.39, 1.33)	.29		
Third quartile	61/100 (61.0%)	0.81 (0.42, 1.53)	.51		
Fourth quartile	68/102 (66.7%)	0.87 (0.43, 1.74)	.69		
Influenza A H1N1—odds ratio for seroconversion					
Log_{10} transformed (as continuous variable, odds ratio per unit rise in PFAA measure)					
Log_{10} PFOA	339/403 (84.1%)	1.51 (0.89, 2.56)	.12		
Log_{10} PFOS	339/403 (84.1%)	1.10 (0.51, 2.37)	.80		
Quartiles (as categorical variable)					
PFOA					
First quartile	83/100 (83.0%)	1			
Second quartile	82/102 (80.4%)	0.74 (0.34, 1.59)	.44		
Third quartile	83/100 (83.0%)	1.11 (0.49, 2.50)	.80		

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	Number Who Seroconvert Following Vaccination (Row%)	Adjusted OR (95% CI)[a]	*p* Value	*p* Value for Heterogeneity Across Quartiles	*p* Value for Trend Over Quartiles
Fourth quartile	91/101 (90.1%)	2.23 (0.90, 5.53)	.08	.07	.05
PFOS					
First quartile	88/104 (84.6%)	1			
Second quartile	81/97 (83.5%)	0.97 (0.44, 2.14)	.94		
Third quartile	82/100 (82.0%)	0.78 (0.35, 1.75)	.55		
Fourth quartile	88/102 (86.3%)	0.94 (0.38, 2.31)	.90	.93	.76
Influenza A H3N2—odds ratio for seroconversion					
Log_{10} transformed (as continuous variable, odds ratio per unit rise in PFAA measure)					
Log_{10} PFOA	261/403 (64.8%)	0.76 (0.51, 1.15)	.19		
Log_{10} PFOS	261/403 (64.8%)	1.17 (0.63, 2.15)	.62		
Quartiles (as categorical variable)					
PFOA					
First quartile	68/100 (68.0%)	1			
Second quartile	68/102 (66.7%)	0.90 (0.48, 1.68)	.75		
Third quartile	69/100 (69.0%)	1.13 (0.59, 2.17)	.71		
Fourth quartile	56/101 (55.4%)	0.62 (0.33, 1.16)	.14	.22	.20
PFOS					
First quartile	65/104 (62.5%)	1			
Second quartile	60/97 (61.9%)	1.08 (0.59, 1.97)	.81		
Third quartile	64/100 (64.0%)	1.10 (0.59, 2.06)	.76		
Fourth quartile	72/102 (70.6%)	1.41 (0.72, 2.78)	.32	.78	.35

[a] *Note*. Adjusted for age (cubic spline), gender, mobility, and history of previous influenza vaccination. Range of PFAA concentrations within quartiles: PFOA: First quartile: 0.25–13.7 ng/ml; second quartile: 13.8–31.5 ng/ml; third quartile: 31.6–90 ng/ml; fourth quartile: 90.4–2140 ng/ml.

[b] PFOS: First quartile: 0.1–5.8 ng/ml; second quartile: 5.9–9.2 ng/ml; third quartile: 9.3–14.5 ng/ml; fourth quartile: 14.7–42.3 ng/ml.

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Table 5

Logistic Regression OR (95% CI) of Seroprotection (HI Antibody Titer ≥ 1:40 Following Vaccination) for Log_{10} Transformed and Quartiles of PFOA and PFOS Concentration (ng/ml) (*n* = 403)

Patient Demographic	Number Are Seroprotected Following Vaccination (Row%)	Adjusted OR (95% CI)[a]	*p*	*p* Value for Heterogeneity Across Quartiles	*p* Value of for TrendOver Quartiles
Influenza type B—odds ratio for seroprotection					
Log_{10} transformed (as continuous variable, odds ratio per unit rise in PFAA measure)					
Log_{10} PFOA[a]	266/403 (66.0%)	1.04 (0.68, 1.60)	.85		
Log_{10} PFOS[b]	266/403 (66.0%)	0.85 (0.44, 1.64)	.63		
Quartiles (as categorical variable)					
PFOA					
First quartile	72/100 (72.0%)	1		.52	.68
Second quartile	66/102 (64.7%)	0.76 (0.40, 1.45)	.41		
Third quartile	68/100 (68.0%)	1.13 (0.57, 2.23)	.73		
Fourth quartile	60/101 (59.4%)	0.77 (0.39, 1.50)	.44		
PFOS					
First quartile	75/104 (72.1%)	1		.63	.50
Second quartile	59/97 (60.8%)	0.67 (0.35, 1.25)	.21		
Third quartile	66/100 (66.0%)	0.82 (0.42, 1.59)	.55		
Fourth quartile	66/102 (64.7%)	0.73 (0.36, 1.47)	.38		
Influenza A H1N1—odds ratio for seroprotection					
Log_{10} transformed (as continuous variable, odds ratio per unit rise in PFAA measure)					
Log_{10} PFOA	385/403 (95.5%)	2.34 (0.91, 6.07)	.08		
Log_{10} PFOS	385/403 (95.5%)	0.93 (0.23, 3.71)	.91		
Quartiles (as categorical variable)					
PFOA					
First quartile	97/100 (97.0%)	1		.04	.02
Second quartile	95/102 (93.1%)	0.74 (0.17, 3.28)	.69		
Third quartile	94/100 (94.0%)	1.59 (0.33, 7.70)	.57		

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Patient Demographic	Number Are Seroprotected Following Vaccination (Row%)	Adjusted OR (95% CI)[a]	*p*	*p* Value for Heterogeneity Across Quartiles	*p* Value of for TrendOver Quartiles
Fourth quartile	99/101 (98.0%)	6.47 (0.91, 45.85)	.06		
PFOS					
First quartile	101/104 (97.1%)	1		.40	.41
Second quartile	90/97 (92.8%)	0.55 (0.13, 2.37)	.42		
Third quartile	97/100 (97.0%)	1.81 (0.32, 10.22)	.50		
Fourth quartile	97/102 (95.1%)	1.26 (0.24, 6.61)	.79		
Influenza A H3N2—odds ratio for seroprotection					
Log_{10} transformed (as continuous variable, odds ratio per unit rise in PFAA measure)					
Log_{10} PFOA	338/403 (83.9%)	0.66 (0.39, 1.12)	.12		
Log_{10} PFOS	338/403 (83.9%)	0.63 (0.26, 1.49)	.29		
Quartiles (as categorical variable)					
PFOA					
First quartile	91/100 (91.0%)	1		.03	.07
Second quartile	82/102 (80.4%)	0.34 (0.14, 0.83)	.02		
Third quartile	80/100 (80.0%)	0.28 (0.11, 0.70)	.01		
Fourth quartile	85/101 (84.2%)	0.39 (0.15, 0.99)	.05		
PFOS					
First quartile	89/104 (85.6%)	1		.36	.24
Second quartile	82/97 (84.5%)	0.85 (0.38, 1.88)	.68		
Third quartile	87/100 (87.0%)	1.09 (0.47, 2.56)	.84		
Fourth quartile	80/102 (78.4%)	0.56 (0.24, 1.28)	.17		

Note. [a]Adjusted for age (cubic spline), gender, mobility, and history of previous influenza vaccination.

Range of PFAA concentrations within quartiles:

[a]PFOA: First quartile: 0.25–13.7 ng/ml; second quartile: 13.8–31.5 ng/ml; third quartile: 31.6–90 ng/ml; fourth quartile: 90.4–2140 ng/ml.

[b]PFOS: First quartile: 0.1–5.8 ng/ml; second quartile: 5.9–9.2 ng/ml; third quartile: 9.3–14.5 ng/ml; fourth quartile: 14.7–42.3 ng/ml.

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Table 6

Self-reported Cold and Flu infections in 12 Months Preceding Questionnaire (*n* = 755)

	PFOA			PFOS		
	Number Reporting Episode in Last 12 Months (%)	**Adjusted OR (95%CI)[a]**	***p* Value**	**Number Reporting Episode in Last 12 Months (%)**	**Adjusted OR (95%CI)[a]**	***p* Value**
OR for any "flu" infection in last 12 months						
Log_{10}-transformed PFOA/PFOS (continuous)	163/755 (21.6%)	0.98 (0.70, 1.38)	.92	163/755 (21.6%)	0.97 (0.58, 1.63)	.91
PFOA/PFOS exposure group (categorical)						
First quartile	45/191 (23.6%)	1		51/193 (26.4%)	1	
Second quartile	37/189 (19.6%)	0.91 (0.55, 1.51)		39/187 (20.9%)	0.83 (0.51, 1.35)	
Third quartile	44/188 (23.4%)	1.30 (0.79, 2.13)		33/190 (17.4%)	0.74 (0.44, 1.24)	
Fourth quartile	37/187 (19.8%)	1.09 (0.65, 1.83)	.57	40/185 (21.6%)	1.20 (0.70, 2.04)	.29
OR for any cold in last 12 months						
Log_{10}-transformed PFOA (continuous)	538/755 (71.3%)	0.83 (0.61, 1.13)	.23	538/755 (71.3%)	0.83 (0.51, 1.34)	.44
PFOA/PFOS exposure group (categorical)						
First quartile	145/191 (75.9%)	1		140/193 (72.5%)	1	
Second quartile	141/189 (74.6%)	1.13 (0.69, 1.83)		146/187 (78.1%)	1.60 (0.99, 2.60)	
Third quartile	131/188 (69.7%)	0.97 (0.60, 1.57)		134/190 (70.5%)	1.20 (0.75, 1.92)	
Fourth quartile	121/187 (64.7%)	0.80 (0.50, 1.29)	.53	118/185 (63.8%)	1.09 (0.67, 1.78)	.24
OR for either a cold or "flu" in last 12 months						
Log_{10}-transformed PFOA (continuous)	554/755 (73.4%)	0.85 (0.62, 1.16)	.31	554/755 (73.4%)	0.90 (0.55, 1.48)	.68
PFOA/PFOS exposure group (categorical)						
First quartile	148/191 (77.5%)	1		145/193 (75.1%)	1	
Second quartile	145/189 (76.7%)	1.21 (0.73, 2.00)		150/187 (80.2%)	1.66 (1.00, 2.75)	
Third quartile	137/188 (72.9%)	1.10 (0.67, 1.81)		137/190 (72.1%)	1.19 (0.74, 1.94)	
Fourth quartile	124/187 (66.3%)	0.84 (0.52, 1.36)	.46	122/185 (66.0%)	1.15 (0.69, 1.91)	.24

[a] *Note*. Adjusted for age (cubic spline) and gender.

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Table 7
Frequency of Cold Infections in 12 Months Preceding Questionnaire (*n* = 755)

Number of Colds Reported in Last 12 Months	Participants Reporting This Frequency of Colds (%)
0	217 (28.7%)
1	404 (53.5%)
2	122 (16.2%)
3	9 (1.2%)
4	3 (0.4%)

	PFOA		PFOS	
	Adjusted OR (95%CI)[a]	LRT[b] for Null vs General Association *p* Value	Adjusted OR (95%CI)[a]	LRT for Null vs General Association *p* Value
OR for frequency of colds in last 12 months				
Log_{10}-transformed PFOA/PFOS (continuous)	0.91 (0.70, 1.19)	.51	0.89 (0.60, 1.33)	.58
PFOA/PFOS exposure group (categorical)				
First quartile	1		1	
Second quartile	0.93 (0.63, 1.38)		1.56 (1.05, 2.31)	
Third quartile	0.95 (0.63, 1.41)		1.05 (0.70, 1.56)	
Fourth quartile	0.90 (0.60, 1.36)	.97	1.10 (0.71, 1.69)	.10

[a] *Note.* Adjusted for age (cubic spline) and gender.

[b] Likelihood-ratio test.